EFFECTS OF SOME COMMON WEEDS FROM PAKISTAN ON PLANT-PARASITIC NEMATODES *IN VITRO* AND POPULATION DENSITIES AND SURVIVAL OF *MELOIDOGYNE INCOGNITA* IN OKRA AND BRINJAL

S.S. Shaukat, I.A. Siddiqui and B. Zarina

Soil Biology and Ecology Laboratory, Department of Botany, University of Karachi, Karachi-75270, Pakistan
*National Nematological Research Centre, University of Karachi, Pakistan**

Summary. Aqueous extracts of the four weed species *Conyza canadensis, Blumea obliqua, Amaranthus viridis* and *Eclipta prostrata* caused substantial mortality of the plant-parasitic nematodes *Xiphinema americanum, Tylenchulus semipenetrans* and *Meloidogyne incognita in vitro*. *Conyza canadensis* and *A. viridis* were the most effective in killing the nematodes while *E. prostrata* was the least effective. Soil amendment with *C. canadensis* or *A. viridis* at the highest concentration (5%) resulted in a significant reduction in soil population densities, root-knot development, and nematode reproductive potential of *M. incognita* in okra and brinjal. Soil amendment with *B. obliqua* at 5% concentration increased plant height of both crops while *A. viridis* at 5% concentration reduced the fresh weight of okra shoots. Ether extracts of the weed species revealed qualitative differences in phenolic compounds. Altogether, thirteen phenolic compounds were isolated from the weeds with *A. viridis* yielding the highest number (6) of such compounds.

Nematodes are devastating parasites of crop plants. Although assessing the magnitude of problem is difficult (Bird and Bird, 2001), based on an extensive international survey (Sasser and Freckman, 1987) it has been estimated that worldwide overall crop yield loss averages 12.3% annually. Of the various plant-parasitic nematodes, several species of *Xiphinema,* dagger nematodes, are serious pathogens of many important crops in Pakistan. The most damaging are members of the *Xiphinema americanum* Cobb group, which are widespread in grape-growing areas of the country. Similarly, *Tylenchulus semipenetrans* Cobb has been found to be the major cause of decline of vigour and yield of citrus in Southern Sindh, Pakistan, and *Meloidogyne incognita* (Kofoid *et* White) Chitw. parasitizes a wide range of plants (Maqbool, 1992).

Nematode control is based on nematicides, which present potential risk to non-target organisms and the environment. In the search for more benign acceptable alternatives to chemicals, the possibilities are being investigated of exploiting nematode-antagonistic plants for the management of plant-parasitic nematodes. Aqueous extracts of various plant species reduced the mobility of *Xiphinema americanum in vitro* (Insunza *et al.,* 2001). Shaukat and Siddiqui (2001a) found that extracts of various weeds from Karachi, Pakistan, caused significant mortality of *M. javanica* (Treub) Chitw. juveniles *in vitro*. Likewise, soil amendment with *Argemone mexicana,* a tropical annual weed, substantially lowered the populations of *M. javanica* in the roots and rhizosphere of tomato plants under greenhouse conditions (Shaukat *et al.,* 2002). The objectives of the present investigation were (i) to determine the effects of aqueous extracts of four weed species on juveniles of the plant-parasitic nematodes *X. americanum, T. semipenetrans* and *M. incognita in vitro*, (ii) to observe the effect of soil amendment with these weed species on nematode soil population densities, root-knot development and nematode reproductive potential in okra and brinjal, and (iii) to assess the phenolic compounds occurring in the weed species, since these compounds often possess nematicidal activity.

MATERIALS AND METHODS

The four common weed species used were *Conyza canadensis* (L.) Cronquist, *Blumea obliqua* (L.) Druce, *Amaranthus viridis* L. and *Eclipta prostrata* (L.) L. They were collected from the experimental field of the Crop Diseases Research Institute, Karachi University Campus. The shoot material of the plants was air-dried in the shade and finely powdered. A 50-g sample of powdered shoot material was soaked in 200 ml of sterile distilled water and left for 72 h at room temperature. The extract was filtered through two layers of Whatman No.1 filter paper and kept at 6 °C prior to use. To avoid microbial contamination of the extract, appropriate quantities of antibiotics (streptomycin and penicillin) were added.

The juveniles of *X. americanum* and *T. semipenetrans* were collected from soils in which lemon [*Citrus limon* (L.) Burm.f.] trees were growing at the National Nematological Research Centre, Karachi University, using Cobb's decanting and sieving technique followed by a modified Baermann funnel technique (Rodríguez-Kábana and Pope, 1981). *Meloidogyne incognita* was originally obtained from a field population and was maintained on tomato (*Lycopersicon esculentum* Mill.). Egg masses were handpicked with sterile forceps and

placed in a beaker containing 50 ml of sterile distilled water. Five days after hatching, the juveniles were collected in a beaker and surface sterilized with 1% $CaOCl_2$ for 30 sec. This inoculum was used for laboratory and glasshouse tests.

Effect of aqueous extract. To assess the effects of aqueous extract of each plant species on mortality of nematodes, 2-ml sample of each extract was transferred to a glass cavity slide and about 21±2, 25±4 or 36±5 juveniles of *X. americanum*, *T. semipenetrans* or *M. incognita*, respectively, were added to each slide. Juveniles kept in sterile distilled water (with the same concentration of antibiotics as in the extracts) served as controls. Preliminary studies had shown that antibiotics, such as streptomycin and penicillin, had no detrimental effect on nematode juveniles. Treatments and controls were replicated ten times and dead nematodes in each cavity slide were counted after 48 h. The experiment was performed twice.

Effect of soil amendment. Powdered shoot material of the test plants was thoroughly mixed with sandy loam soil (72% sand, 17% silt and 11% clay; pH 8.1; organic matter content 0.3%) to make 30 or 50 g/kg (3 or 5% w/w) concentrations and put into 8-cm-diam. plastic pots at 400 g/pot. Unamended soil served as control. The pots were watered daily to promote microbial activity and thereby initiate decomposition of the plant tissues. Three weeks after setting up the pots, four presoaked okra (*Abelmoschus esculentus* L.) seeds were sown in 54 pots and, after germination, were thinned to three seedlings per pot. In another 54 pots, three brinjal (*Solanum melongena* L.) seedlings (about 8 cm tall and at the two-leaf stage), raised in steam sterilized soil, were planted in each pot. The seedlings were allowed to establish for one week before soil in each pot was inoculated by adding a total of 2,000 freshly hatched juveniles (< one week-old) of *M. incognita* in four small holes made in the soil around each plant. Treatments and controls were replicated six times and randomized within blocks on a glasshouse bench maintained at 25-30 °C.

Plants were harvested 52 days after transplanting and growth parameters, including plant height and fresh weight of shoot and root, were recorded. The number of galls and egg masses produced on the entire root system was counted using a hand lens. Root-knot nematodes were extracted from the soil (250 cm^3) using a modified Baermann funnel technique and counted (Rodríguez-Kábana and Pope, 1981). The experiment was performed twice.

Since *C. canadensis* suppressed *M. incognita* in both okra and brinjal, even at the low (3%) application rate, another experiment was designed to determine the least concentration at which galling induced by the nematode is inhibited. In addition to the concentrations used in the previous experiment, 5, 10 and 20 g/kg of soil (0.5, 1 and 2% w/w) were also used. The rest of the procedure was the same as above, except that only brinjal was used as the test crop and only the number of galls per root system was recorded.

Assessment of phenolic compounds. Ether extracts of the weeds were evaporated to dryness and the residue, dissolved in 2 ml of 80% ethanol, was loaded on to silica gel F_{254} thin layer chromatography plates. The chromatograms were developed in either acetic acid-chloroform 1:9 v/v or n-butanol-acetic acid-water 4:1:5 (top layer) v/v/v by ascending chromatography using reference phenolic compounds. Phenolic compounds were detected using ferric chloride-ferric cyanide reagent and UV light (Harborne, 1973).

All data were subjected to analysis of variance (ANOVA) and factorial analysis of variance (FANOVA), depending upon the experimental design, followed by Fisher's least significant difference (LSD) test using STATISTICA ver. 5.0 software (1995; Statsoft Inc., Tulsa, Oklahoma, USA). In the case of repeated experiments, analysis was performed separately and then on the pooled data. An F-test was conducted to determine whether the results from different trials were significantly different and to determine if pooling the data was warranted. Percentage data were transformed to arcsine prior to analysis.

RESULTS AND DISCUSSION

Aqueous extracts of four weed species, *C. canadensis*, *B. obliqua*, *A. viridis* and *E. prostrata*, caused significant ($P < 0.05$) mortality of the plant-parasitic nematodes *X. americanum*, *T. semipenetrans* and *M. incognita* (Table I). The weed species exerted a differential impact on different nematode species. For instance, *C. canadensis*

Table I. Effects of shoot extracts of four weed species on the mortality of *Xiphinema americanum, Tylenchulus semipenetrans* and *Meloidogyne incognita* 48 h after exposure.

Weed species	Mortality %		
	X. americanumn	*T. semipenetrans*	*M. incognita*
Control	2	5	6
Conyza canadensis	31	66	52
Blumea obliqua	19	31	23
Amaranthus viridis	37	52	41
Eclipta prostrata	27	34	38
$LSD_{0.05}$	15	22	18

caused most mortality of the juveniles of *T. semipenetrans* and *M. incognita* while *A. viridis* induced greater mortality of *X. americanum*. With respect to nematicidal activity, regardless of the nematode species, *B. obliqua* was least effective.

Soil amendment with powdered shoot material of *C. canadensis* or *A. viridis* reduced nematode soil population densities in okra at both of the concentrations (3 and 5%) and *C. canadensis* in brinjal at the 5% concentration (Table II). Application of *C. canadensis* at 5% resulted in a significant ($P < 0.05$) inhibition of root-knot development in okra while *C. canadensis* at both the concentrations and *A. viridis* at 5% inhibited ($P < 0.05$) galling in brinjal. *Conyza canadensis* at 5% inhibited egg mass production in both okra and brinjal while *A. viridis* at 5% reduced egg mass production in brinjal. The suppressive effects of weeds, particularly *C. canadensis* and *A. viridis,* has been related directly to the release of compounds toxic to plant-parasitic nematodes, and indirectly to a change in microbial community structure in the rhizosphere (Shaukat and Siddiqui, 2001c) and roots of host plants antagonistic to such nematodes. Although our results suggested that toxic principles were released from the weed species in the *in vitro* study, alterations in microbial community structure and composition following soil amendments with these weeds need further investigation.

Soil application of *B. obliqua* at both concentrations enhanced plant height of okra, while *B. obliqua* at 5% increased plant height of brinjal (Table III). Interestingly, *A. viridis* at 5% reduced shoot weight of okra. *Conyza canadensis* at 5% reduced the fresh weight of roots of okra plants while *A. viridis* at 5% reduced root weight of brinjal. This reduction in fresh weight may be due to the release of phenolic compounds in the crop rhizosphere.

When a series of concentrations (0.5, 1, 2, 3 and 5%) of *C. canadensis* shoot material in soil was tested, it was found that the galling rate was reduced significantly ($P < 0.05$) only at the 3 and 5% concentrations, though there was a slight (non-significant) decrease at 2% as well (data not presented).

The assessment of phenolic composition of the four weed species revealed marked qualitative differences (Table IV). Altogether, thirteen phenolic compounds were detected from the four weed extracts. *Conyza canadensis* and *A. viridis* contained five and six phenolic compounds, respectively, while *B. obliqua* had the least number, with three phenolic acids. Phenolic compounds are known to possess nematicidal activity. Shaukat and Siddiqui (2001b) demonstrated that phenolics such as *p*-hydroxybenzoic acid, *p*-coumaric acid and caffeic acid cause substantial mortality of *M. javanica* juveniles *in vitro*. It is, however, not known whether the other phenolics found here also possess such nematicidal potential. Thus, the nematicidal potential of the weeds tested can in part be attributed to their phenolic contents. Compounds other than phenolics that could have nematicidal potential were not tested but could be important in imparting nematicidal potential to the weeds.

Table II. Influence of soil amendment with powdered shoot material of four plant species on nematode population densities in soil, root-knot and egg mass development due to *Meloidogyne incognita* on okra and brinjal.

Treatment	Conc. (%)	Nematode populations in 250 cm^3 soil		Galls per root system		Egg masses per root system	
		Okra	Brinjal	Okra	Brinjal	Okra	Brinjal
Control	-	1430	2355	62	126	27	77
Conyza canadensis	3	1155	2245	36	98	16	65
	5	945	1870	29	84	5	48
Blumea obliqua	3	1510	2305	55	103	22	69
	5	1275	2250	43	110	13	61
Amaranthus viridis	3	1215	2215	41	117	10	72
	5	1050	2045	38	95	8	52
Eclipta prostrata	3	1470	2585	51	115	30	84
	5	1325	2535	45	104	18	63
$LSD_{0.05}$							
Treatment		188		28		21	
Concentration		165		22		18	

Table III. Influence of soil amendment with powdered shoot material of four plant species on growth of okra and brinjal plants.

Treatment	Conc. %	Plant height (cm)		Shoot weight (g)		Root weight (g)	
		Okra	Brinjal	Okra	Brinjal	Okra	Brinjal
Control	-	26.4	14.6	3.3	1.9	2.1	2.3
Conyza canadensis	3	28.9	15.2	3.6	2.4	1.8	2.2
	5	27.7	11.3	2.9	1.8	1.3	1.7
Blumea obliqua	3	31.1	17.7	3.2	2.4	1.8	2.0
	5	33.4	19.4	3.7	2.8	1.5	1.9
Amaranthus viridis	3	24.1	12.9	2.7	1.7	2.0	2.1
	5	22.7	11.1	2.0	1.4	1.8	1.6
Eclipta prostrata	3	29.8	16.4	3.8	2.1	1.6	2.2
	5	27.3	15.2	3.4	2.4	2.0	1.8
$LSD_{0.05}$							
Treatment		4.2		1.1		0.7	
Concentration		3.1		0.7		0.5	

Table IV. *Rf*-values of phenolic principles in ether fractions of aqueous extracts of four weed species and their reaction under UV light. The solvent systems were acetic acid:chloroform (1:9 v/v)[a] or *n*-butanol:acetic acid:water (4:1:5 v/v/v)[b].

Compound	*Rf* values				UV light observation
	C. canadensis	*B. obliqua*	*A. viridis*	*E. prostrata*	
p-hydroxybenzoic acid[a]	-	-	55.23	-	Blue
p-coumaric acid[b]	-	93.42	-	-	None
Salicylic acid[a]	-	-	90.88	91.22	Blue
Vanillic acid[a]	84.14	-	83.03	-	Light blue
Syringic acid[a]	79.57	-	78.74	-	Blue
Catechol[a]	37.24	-	-	35.82	Dull blue
Gallic acid[a]	6.20	-	-	-	Blue
Gentisic acid[a]	-	35.38	-	-	Light-blue
4-methyl-resorcinol[a]	-	25.24	-	24.85	Violet-blue
Protocatechuic acid[a]	-	-	20.10	-	Blue
Pyrogallol[a]	-	-	7.92	-	Bluish-brown
Caffeic acid[b]	80.16	-	-	-	Bright-blue fluorescent
Unknown[a]	-	-	-	95.62	Brown

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Accepted for publication on 29 March 2004.